# Transcription and DNA Damage: A Link to a Kink

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Living organisms are constantly exposed to a variety of naturally occurring and man-made chemical and physical agents that pose threats to health by causing cancer and other illnesses, as well as cell death. One mechanism by which these moieties can exert their toxic effects is by inducing modifications to the genome. Such changes in DNA often result in the formation of nucleotides not normally found in the double helix, bases containing covalent chemical alterations, single- and double-strand breaks, and interstrand and intrastrand cross-links. When these lesions are present during replication, mutations often result in the newly synthesized DNA. Likewise, when such damage occurs in a gene, transcription elongation, and hence expression, can be adversely affected because of pausing or arresting of the RNA polymerase at or near the altered site; this could result in the synthesis of a defective RNA molecule. It has become increasingly clear that transcription and DNA damage are intimately linked, since the removal of certain adducts from the genome is highly dependent on their location: When such lesions are present on the transcribed strand of actively expressed genetic loci, they are better cleared from that strand when compared to the complementary DNA or other quiescent regions. This process is called transcription-coupled DNA repair, and it modulates the mutagenic spectrum of many DNA-damaging agents. Furthermore, based upon evidence from systems in which it is absent, this process has a profound effect on ameliorating the adverse consequences of exposure to many environmentally relevant genotoxins. The precise cellular pathway that mediates the preferential clearance of DNA damage from active genetic loci has not yet been established, but it appears to be effected by a repertoire of proteins that are also involved in other DNA repair pathways and transcription as well as some factors that might be unique to it. Because a cellular process as indispensable as gene expression can be thwarted by the presence of DNA damage, an understanding of the mechanism underlying transcription-coupled DNA repair is relevant to the continued discernment of how environmental genotoxins endanger human health. — Environ Health Perspect 105(Suppl 1):145–153 (1997)

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## Introduction

Many hazardous environmental agents exert their toxic effects on humans and other organisms by inducing modifications to the genome; included among these agents are numerous natural and man-made chemical compounds as well as several types of radiation. The damage produced in DNA by these genotoxins consists of single- and double-strand breaks, modified bases and phosphate groups, and interstrand and intrastrand cross-links, among others (*1,2*). When these alterations remain in the double helix, replication can be blocked or impeded, mutations to the genome can result when replication does occur, and gene expression can be compromised due to improper transcription, potentially leading to cellular transformation or death (*2,3*). It is critical that such damage be removed to ensure fidelity during DNA synthesis and to enable the cell to continue appropriate RNA synthesis.

Broadly defined, DNA repair refers to the collected pathways in a cell that assist in maintaining genomic integrity by removing inappropriate bases and other possible deleterious lesions from DNA. Numerous mechanisms have evolved to this end: *a*) nucleotide excision repair (NER) (*2,4–6*), *b*) base excision repair (BER) (*2,7,8*), *c*) mismatch repair (MMR) (*2,9*), and *d*) direct reversal of the damage, in which no incision is made in the backbone of the DNA (*2,10,11*). Overlap among these pathways exists in terms of the types of damage removed by each.

An important component of the DNA repair process that is involved in clearing the genome of damage is transcription-coupled DNA repair (TCR). This phenomenon is characterized by more rapid removal of certain modified bases from the transcribed strand of actively expressed genes when compared to silent DNA (*3,12,13*). It has been proposed that TCR might exist to ensure that transcription can readily continue following a genotoxic assault, thus providing a means for producing transcripts essential for continued cell survival (*3*). A corollary to this notion is that lesions in DNA that block or inhibit the progression of RNA polymerases are precisely those that are subject to TCR (*3,13*). Hence, the process of transcription is linked to DNA damage in that it can influence the clearance rate of certain lesions formed in the genome following exposure to many environmentally relevant genotoxins.

Several questions lie at the heart of current studies designed to understand TCR: *a*) What lesions are subject to it? *b*) How do such alterations to DNA impede or block the progression of RNA polymerases? *c*) What is the actual mechanism by which preferential clearance of DNA adducts is achieved, and *d*) which organisms exhibit the phenomenon? The first question concerns the classes of DNA damage that are cleared preferentially from actively expressed genetic domains and is addressed in Table 1. This article will focus more on issues *b* and *c*—the matter of DNA damage posing blocks to transcription and

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Abbreviations used: AAF, acetylaminofluorene; AF, aminofluorene; BER, base excision repair; BPDE, benzo[*a*]pyrenediol epoxide; CPD(s), cyclobutane pyrimidine dimer(s); CS, Cockayne's Syndrome; dG, deoxyguanosine; *dhfr*, dihydrofolate reductase; dT, deoxythymidine; HNPCC, hereditary nonpolyposis colorectal cancer; mfd, mutation frequency decline; MMR, mismatch repair; NER, nucleotide excision repair; TCR, transcription-coupled DNA repair; UV, ultraviolet; XP, xeroderma pigmentosum.

**Table 1.** Genotoxic agents and their relationships to TCR.

| Genotoxic agents producing DNA damage | Selected references[a] |
|---|---|
| Subject to TCR | |
| UV Radiation | (*14–16*) |
| Benzo[*a*]pyrene | (*17*)[b] |
| Benzo[*c*]phenanthrene | (*18*) |
| *N*-Ethyl-*N*-nitrososurea | (Sitaram et al., unpublished observations) |
| CC-1065 | (*19*) |
| Aflatoxin $B_1$ | (*20*) |
| Cisplatin | (*21*) |
| Oxidizing agents/ ionizing radiation | (*22*) |
| Psoralen interstrand DNA cross-links | (*23,24*) |
| Not subject to TCR | |
| Dimethyl sulfate | (*25*) |
| Methyl methanesulfonate | (*26*) |
| *N*-Methyl-*N*-nitrosourea | (*26*) |
| *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine | (*26*) |
| Aminofluorene | (*27*) |
| Psoralen monoadducts | (*24*) |
| Benzo[*a*]pyrene | (*28*)[b] |

[a]For a more complete list of references concerning TCR of DNA adducts, see Friedberg et al. (*2*).
[b]Benzo[*a*]pyrene has been reported to produce damage that is subject to TCR in one case but not cleared by this pathway in another. Importantly, the investigators used different cell lines and different genetic loci in each, indicating that TCR of DNA damage is a function of cell type and the target locus.

the mechanism of TCR. Transcription-coupled DNA repair in mammalian systems will be emphasized, with studies concerning this type of repair in *Escherichia coli* being described because of the attributes of this organism that make it a useful model for understanding the process. Spatial constraints do not permit descriptions of TCR in yeast and other important systems; information concerning the process in these organisms can be found in several current texts and reviews (*2,29*). Also, the involvement of DNA repair during organismal development, an important issue due to the high levels of replication and transcription during maturation, will not be addressed (*30*).

Neither the precise series of events nor all of the proteins required during TCR have been fully elucidated; however, recent studies suggest that components of several DNA repair pathways are involved, with the presence of additional factors being necessary to link transcription to the removal of damage from active genes. Therefore, better understanding of TCR can be achieved by considering the involvement of individual repair pathways and coupling factors in the process. To that end, a summary of what is known about the effect of modified bases in DNA on pausing and arresting RNA polymerases during transcription elongation will be undertaken; this will be followed by brief discussions of individual DNA repair pathways, with each section containing a description of what is known about that particular pathway's relationship to TCR. Finally, a model describing a potential mechanism for TCR will be presented.

## Transcription and DNA Damage

### Gene Expression and Transcription-coupled DNA Repair

An important question concerning TCR now needs to be considered: Is transcription actually necessary for TCR of an adduct, or is the mere presence of a lesion in the transcribed strand of a gene sufficient for its preferential removal from the region? Studies evaluating TCR in *E. coli* have made use of the lac operon as a target locus to address this issue. Following exposure to ultraviolet (UV) light, cyclobutane pyrimidine dimers (CPDs) were removed from the transcribed and nontranscribed strands of the uninduced *lacZ* gene at virtually identical rates, with 50% of the adducts being cleared in 20 min. When an identical experiment was performed using *E. coli* previously exposed to an agent that induces the *lac* operon, the rate of repair in the transcribed strand of the *lacZ* gene rose approximately 10-fold, such that it was cleared of 50% of the damage in about 2 min; repair in the nontranscribed strand remained the same as that seen in the uninduced state. These results clearly demonstrate the need for active gene expression for TCR to occur in *E. coli* (*31*).

For mammalian cells, several pieces of evidence suggest that active transcription by RNA polymerase II is needed for TCR. When cells are exposed to α-amanitin, a drug that inhibits RNA polymerase II, TCR of CPDs is abolished (*32*). Interestingly though, rRNA genes, which are transcribed by RNA polymerase I, are not subject to TCR (*33*). These data, in conjunction with those from *E. coli*, insinuate that the rapid repair of lesions from transcribed DNA requires that the locus in question be actively transcribed by certain RNA polymerases and that the region encodes mRNA.

### DNA Damage: RNA Polymerase Pauses and Arrests

The notion that TCR is activated by lesions in DNA that impede transcription relies on understanding the effect of DNA modifications on RNA synthesis. A gene is comprised of both an actual transcription unit and regulatory regions that assist in controlling its expression. Theoretically, damage located at any position within a gene could impede transcription. A lesion could cause the polymerase to pause, with eventual bypass occurring, or pose a block to its progression, causing it to arrest and stop elongation. These events could occur at any of the three basic stages associated with the process—initiation, elongation, and termination. If an adduct were present in the regulatory region of the gene and it prevented the formation of an initiation complex, expression of that gene would be seriously impaired. Similarly, if the adduct were in the transcription unit and RNA polymerase progression were to be paused or arrested, faulty transcripts could result, jeopardizing vital cellular processes; it is this situation that has been hypothesized to provide part of the link between DNA damage and transcription (*3*).

Studies concerning the effect of base modifications in DNA on RNA synthesis by RNA polymerases are much less well developed than those for DNA polymerase effects on both processivity and base misincorporation. Recently, however, descriptions of the behavior of a variety of RNA polymerases at adducts placed at specific sites in a DNA template have been reported. Such a technique has the advantage of permitting the investigator to know the precise nature and position of the DNA modification being examined. Lesions derived from radiation and chemicals have been explored in this way, and a summary of some of the important findings is essential for analyzing the role of blocked transcription in TCR.

Early experiments concerning the ability of DNA damaged by UV radiation to block transcription were described by Sauerbier and Hercules (*34*). Recently, investigations using thymine-thymine CPDs placed site-specifically in a DNA template containing the major late promoter of adenovirus showed that these lesions are strong blocks to the progression of rodent RNA polymerase II; furthermore, the lesion must be present on the template strand for the polymerase to stall. It was also shown that elongation factor SII ($TF_IIS$) can induce cleavage of the nascent transcript at the site

of the dimer, but the process does not enhance the bypass of the lesion (*35*). These studies are quite significant because they address the issue of the effect of transcription elongation factors on lesion bypass; in fact, as more such factors are discovered, consideration of their role in RNA synthesis past DNA adducts will be important.

Studies concerning the bypass of site-specific chemical adducts in DNA have also been undertaken. Psoralen-dT lesions present as either a monoadduct or diadduct efficiently block transcription by T7 RNA polymerase and *E. coli* RNA polymerase (*36,37*); acetylaminofluorene-dG (AAF-dG) and aminofluorene-dG (AF-dG) adducts inhibit transcription by both T7 RNA polymerase and RNA polymerase III (*38,39*); and benzo[*a*]pyrenediol epoxide-dG (BPDE-dG) lesions impede transcription by T7 RNA polymerase (*40*). It is important to understand that, unlike CPDs, many of these adducts do not pose absolute blocks to the progression of an RNA polymerase. For BPDE lesions, the stereochemistry of the actual $N^2$-guanine adduct profoundly affects the ability of bypass to occur (*40*). Hence, if a stalled RNA polymerase complex were a requisite event for the TCR of an adduct, certain modifications to DNA could escape this pathway and be repaired by alternate mechanisms or remain in the gene. Also, the role of an elongation factor such as SII might be increasingly important for adducts that act as pause sites rather than arrest signals; of course, the validity of this needs further testing.

Another important aspect of transcriptional bypass of DNA adducts is the notion of base misincorporation in mRNA, an event that could change the protein encoded by the transcript; likewise, the dissociation of a nascent mRNA at the site of an adduct results in a truncated transcript, an event that would lead to synthesis of an incomplete protein or possibly no protein. It is precisely these possibilities that might form the underlying reason that preferential clearance of lesions from actively transcribed DNA exists: TCR supplies a rapid and efficient means of removing adducts from active genes, which protects the innate integrity of the mRNA being produced (*3*). Interestingly, though, full-length transcripts resulting from lesion bypass of AF-dG, AAF-dG, and BPDE-dG lesions by T7 RNA polymerase contain the correct nucleotide sequence, but truncated transcripts due to the RNA polymerase stalling in the presence of BPDE-dG adducts almost always end with an incorrect base (Choi et al., unpublished observations). Such data suggest that the ability of a lesion on the transcribed strand of an actively expressed genetic locus to impede the progression of RNA polymerase is a function of several parameters—the stereochemistry of the adduct, its inherent ability to alter the processivity of the polymerase, and the actual base incorporated into the nascent RNA at or near the lesion—and might be modulated by transcription elongation factors.

## Transcription-coupled DNA Repair

### The Role of Coupling Factors

An unsolved problem in the field of DNA repair concerns the composition of the pathway that performs TCR once RNA elongation is impeded by a lesion. Likewise, a question of great importance to the TCR mechanism concerns the existence of factors that might somehow sense a stalled transcription complex, either causing the RNA polymerase to dissociate, thus making the adduct available for repair, or actually summoning the repair machinery to the site of damage, or both. In *E. coli*, a coupling factor that links NER to TCR has been identified; it is the product of the mutation frequency decline (*mfd*) gene and is essential for TCR of CPDs (*41,42*). This particular protein causes RNA polymerase to dissociate, and it interacts with proteins that are part of NER and draws them toward the site of damage. Interestingly, the Mfd protein contains helicase motifs, but it is not a helicase.

In human cells, genetic loci that are defective in Cockayne's Syndrome (CS) encode proteins that are likely candidates for being TCR coupling factors. There are several complementation groups for CS, two unique ones referred to as A and B and three which overlap with another repair deficiency disease called xeroderma pigmentosum (XP) (*2,43*). Cells derived from patients with CS are hypersensitive to UV irradiation, but repair of CPDs does occur in the genome overall (*2,44*). What is absent from CS cells is the ability to perform TCR (*45*). Two CS genes have been cloned; one complements CS-A cells and one complements CS-B cells (*46,47*). The CS-A and CS-B proteins interact with one another; additionally, the CS-A protein can bind to p44 protein, a subunit of the human RNA polymerase II transcription factor H ($TF_{II}H$), suggesting that the CS gene products might be involved in transcription. CS is characterized by a significant lag time in the recovery of RNA synthesis following exposure of the cells to certain genotoxins, perhaps due to the slow removal of adducts from transcriptionally active regions. Hence, the CS-A and CS-B proteins may act as coupling factors for human TCR or might effect changes in chromatin structure at sites of transcription, enabling these loci to be better repaired (*2,33,46,47*).

While the identification of coupling factors that link a stalled transcription complex, DNA damage, and repair machinery has been a great boon to understanding TCR, the precise composition of the repair pathway involved remains to be elucidated. Indeed, the explicit nature of the pathway that elicits TCR is not simple; it appears to involve components of several repair pathways in the cell, each of which may play a distinct role in the process.

### The Role of Nucleotide Excision Repair

Nucleotide excision repair is a repair pathway that removes a wide variety of DNA lesions from the genome; it appears to recognize distortions in the double helix and, therefore, possesses a broad specificity for the types of damage that are cleared by it (*2,4,6*). In *E. coli*, where NER is best understood, the actual incision process at the damaged site is a function of the products of the *uvrA, uvrB,* and *uvrC* genes (*6*). Recognition is accomplished by a protein complex consisting of a UvrA homodimer associated with a UvrB monomer—$UvrA_2$·UvrB. Upon adduct recognition, the UvrA proteins dissociate, and UvrC protein binds, creating an exinuclease that incises the damaged strand on either side of the lesion such that during NER a roughly 12-base segment of DNA containing the damage is removed. The product of the *uvrD* gene, which is helicase II, is essential for the removal of the damaged oligonucleotide and turnover of the UvrB and UvrC proteins (*2,4,6*).

Numerous mutant strains of *E. coli* exist in which functional NER is absent. Such *uvrA*$^-$, *uvrB*$^-$, and *uvrC*$^-$ cells have also been used to investigate the role of NER in TCR in *E. coli*; in these strains, less than 10% of the CPDs were removed from each strand of the *lacZ* gene following exposure to UV radiation, indicating that an intact NER complex not only is required for the general removal of CPDs from these cells but also is indispensable for TCR (*48*).

The clearance of UV-induced CPDs from mammalian cells by NER is vastly

more complex than the equivalent pathway in *E. coli*; it is also less well understood. In human cells, at least 30 gene products are required for functional NER to occur; of this number, 7 were identified partly by studying cells derived from patients afflicted with XP, which is characterized by deficient NER and heightened sensitivity to UV radiation (*2,4,5,49*). Individuals with XP are prone to a variety of pathological conditions, including skin cancer and neurological aberrations. The seven XP complementation groups are labeled A through G, and each exhibits a different phenotypic sensitivity to UV light. For example, XP-A cells do not repair CPDs and are very sensitive to the lethal effects of UV radiation; in contrast, XP-C cells exhibit a modest level of dimer clearance and are less sensitive to the detrimental effects of UV exposure (*49*). A description of some of the genes and the proteins involved in human NER is summarized in Table 2; the function of each is also listed when it is known.

TCR has been observed in mammalian cells derived from both rodents and humans, but the features of the process are different in each case (*14–16*). In rodent cells, the clearance of CPDs is manifested as a high level of repair from the transcribed strand of active genes, with virtually no clearance of these lesions from the nontranscribed counterpart (*16*). In contrast, human cells exhibit repair in both strands of active genes, albeit the rate of repair is faster in the transcribed strand (*16*). In terms of TCR and its relationship to NER, an important issue concerns which of the particular gene products responsible for NER are involved in biased clearance of DNA lesions from human cells. As mentioned, the severest form of XP is manifested in complementation group A. Cells derived from these patients cannot repair CPDs found in the overall genome; they also cannot perform TCR of these adducts, indicating that recognition by the XP-A protein of the actual CPD or the helical distortion is necessary for the transcription-coupled clearance of these adducts (*50,59*).

Two of the gene products that take part in NER, the XP-B and XP-D proteins, also participate as elements of transcription factor $TF_{II}H$ in the cell and possess helicase activity (*51,53,54,60*). It is important to understand that these two proteins play a part in NER as part of $TF_{II}H$ (*61*). In other words, $TF_{II}H$ has a dual function in cells: It acts as a transcription factor and as a component of NER, perhaps by interacting with different sets of proteins in each case. XP-D cells are slightly less sensitive to UV radiation than XP-A cells, and they do remove CPDs to a low but significant extent; however, TCR of these lesions does not occur in most XP-D mutant cells (*2,49,59*). This suggests that the $TF_{II}H$ factor is needed for TCR by participating in NER to clear the lesion; any additional role it plays in the actual coupling process remains to be established. The requirement for the XP-B product in TCR has not yet been evaluated in a cell system, but assuming XP-D protein is involved in biased clearance of adducts via its presence in $TF_{II}H$, it could be predicted that XP-B would also be involved. There is an important caveat associated with the study of TCR in XP-B cells: All identified cases of this XP complementation group overlap with CS, which could make the interpretation of the results somewhat difficult (*2*).

The role of the XP-C protein in NER and TCR is particularly intriguing. XP-C cells are more resistant to the detrimental effects of UV radiation than XP-A, XP-B, and XP-D cells; they also repair CPDs to a small but significant extent (*2,49*). Furthermore, the clearance of CPDs from XP-C cells is limited to regions associated with gene expression, suggesting that these cells are capable of clearing damage from expressed regions of DNA but have limited or no capability to clear the damage from quiescent domains or heterochromatin (*62–64*). Indeed, clearance of CPDs from the transcribed strand of active genes in XP-C cells has been shown to occur (*65*). The *XP-C* gene has been cloned (*52*), but the actual function of the XP-C protein is not clear.

The role of XP-E protein in NER and TCR is not clear, but it does bind to UV-damaged DNA (*55*). Of all the XP complementation groups, XP-E cells are the least sensitive to the lethal effects of exposure to UV light (*2,49*). Whether or not XP-E protein is essential or dispensable during TCR of CPDs or other DNA lesions is not known. Interestingly, XP-E protein is not required for NER in a cell-free system (*2*).

The XP-F protein, in association with ERCC1, and XP-G protein are responsible for incising the DNA on the 5′ and 3′ sides of the lesion, respectively, causing the release of a roughly 29-base fragment of DNA containing the damage (*2,4,6,56,57*). TCR of CPDs requires the XP-F product; however, the need for XP-G protein has not yet been examined (*59*). These results suggest that the actual incision of the damaged DNA during TCR of CPDs requires the same endonucleolytic proteins used during NER of CPDs.

In summary, then, functional NER is necessary for the TCR of CPDs in both bacteria and mammalian cells. Furthermore, studies concerning the specific repertoire of NER proteins in human cells needed for TCR indicate that XP-A, XP-D, and XP-F gene products are required; the remaining NER proteins might also be necessary, but direct experimental data proving this assertion are not yet available. Perhaps the most curious of all the XP proteins in terms of TCR is the XP-C gene product; cells that lack this gene product can perform TCR but cannot execute general NER in quiescent regions of the genome.

A final critical issue that needs to be addressed in terms of TCR is related to the ability to clear chemical adducts in a biased fashion via NER. As illustrated in Table 1, numerous lesions other than those formed following exposure to UV light are subject to TCR. This poses a fundamental question: Is the same set of proteins needed to clear CPDs via TCR also needed for the biased removal of alternate lesions such as

**Table 2.** Genes and their products involved in NER in human cells.

| XP Complementation group | Human gene[a] | Proposed protein function | References |
|---|---|---|---|
| A | *XP-A* | DNA damage recognition | (*50*) |
| B | *XP-B/ERCC3* | Helicase; part of $TF_{II}H$ | (*51*) |
| C | *XP-C* | Part of transcription factors | (*52*) |
| D | *XP-D/ERCC2* | Helicase; part of $TF_{II}H$ | (*53,54*) |
| E | Not cloned | Binds damaged DNA | (*55*) |
| F | *ERCC4* | Nuclease | (*56*) |
| G | *XP-G/ERCC5* | Endonuclease activity | (*57*) |

[a]The *ERCC* genes were identified as those human genes that complemented NER defects in mutant rodent cells; indeed, *ERCC* is an abbreviation for excision repair cross-complementing. The genes that are labeled as *XP-A* and so on are those that were discovered by transfecting human DNA into cells belonging to differing *XP* complementation groups. There is also an *ERCC1* gene whose product does not correct any of the *XP* phenotypes (*58*). A thorough table describing these genes, their chromosomal locations, and rodent, yeast, and fly homologues can be found in Friedberg et al. (*2*).

those formed by exposure to ionizing radiation or chemical compounds? While NER is necessary for TCR of UV-induced dimers, it might not be needed for the biased clearance of all DNA adducts. For example, it has been shown that TCR of ionizing radiation-induced DNA damage occurs in certain cells defective in NER as well as in repair-proficient human cells; this might be a function of the role of BER in TCR (*22*).

## The Role of Base Excision Repair

Another pathway responsible for removing damage from DNA is BER. This mechanism for clearing lesions involves the recognition and removal of certain modified or unmodified bases from the genome by enzymes referred to as glycosylases. Their mode of action involves removal of the target base as the initial step in its clearance (*2,7,8*). Following removal of the target moiety, subsequent action by an abasic endonuclease, which may or may not be an innate activity of the glycosylase itself, cleaves the sugar phosphate backbone. The nicked DNA that is generated is a substrate for exonucleases, and repair is completed by gap-filling by a DNA polymerase and ligation of the newly synthesized DNA to the contiguous DNA (*2,7,8*).

There are numerous examples of DNA damage that is repaired by BER, two of which include *N*-alkylpurines and thymine glycols. The former are removed by 3-alkyladenine–DNA glycosylase, and the latter are cleared by thymine glycol–DNA glycosylase (*2,7,8*). As mentioned, the subsequent endonucleolytic cleavage is sometimes associated with the glycosylase activity, as is the case for thymine glycol–DNA glycosylase, or it can be independent of the base removal step, as is true for 3-alkyladenine–DNA glycosylase. An important issue concerning TCR is whether glycosylase-mediated clearance of damage exhibits a more rapid repair that is associated with the transcribed strand of active genes. Evidence suggesting that this is the case has been reported (*22*). It has been shown that thymine glycols generated in NER- and BER-proficient human cells following exposure to ionizing radiation are cleared in a biased fashion from the transcribed strand of an expressed metallothionein gene. Furthermore, TCR of thymine glycols is still present in the same locus in cells where BER is operating but where NER is absent, suggesting that the thymine glycol–DNA glycosylase is indeed coupled to TCR.

The evidence that thymine glycol repair is linked to TCR via BER leads directly to consideration of whether such coupled repair is found for other lesions that are normally cleared by BER. Experiments testing for biased clearance of *N*-methylpurines from the dihydrofolate reductase (*dhfr*) gene of Chinese hamster ovary B11 cells show no preferential clearance of these adducts from the transcribed strand of this locus regardless of the source of methylation damage (*25,26,66*). Furthermore, when 7-methylguanine and 3-methyladenine repair rates are determined individually for each of the strands of the *dhfr* gene, no TCR is observed in either case. This is an important observation because 3-methyladenine impedes transcription; hence, these data indicate that an interruption to RNA synthesis is not the sole factor responsible for summoning TCR (*26*). Interestingly, the clearance of *N*-ethylpurines shows a strong bias toward the transcribed strand of the *dhfr* gene in cells where NER is intact; this preferential clearance is not seen in cells lacking functional NER, where the observed repair is presumably executed by BER. These results indicate that BER is not coupled to TCR, at least in the case of ethylated purines (Sitaram et al., unpublished observations). Clearly, there would be benefits from investigating *N*-alkylpurine repair in cells lacking the 3-alkyladenine–DNA glycosylase; homozygous mutant mouse cells have recently been characterized that lack this activity, which now makes such experiments feasible (*67*).

Several possible explanations for the contrasting data concerning the preferential clearance of thymine glycols and *N*-methylpurines by BER can be devised. These two glycosylases are quite different, not only in terms of substrate recognition but because thymine glycol–DNA glycosylase actually contains an associated abasic endonuclease activity, whereas 3-alkyladenine–DNA glycosylase does not possess such a feature. There is the possibility that the thymine glycol glycosylase is coupled to transcription, but 3-alkyladenine–DNA glycosylase is not part of TCR. A second possible source of the difference could lie in the fact that thymine glycols are substrates for both NER and BER in eukaryotic cells, whereas *N*-methylpurines are cleared primarily by BER (*6*); however, this does not account for the *N*-ethylpurine data. A third viable explanation might be that thymine glycols are blocks to transcription, a fact that would make them subject to TCR; in contrast, 7-methylguanines, which constitute 80% of the *N*-methylpurines, do not appear to inhibit transcription and would not be predicted to be removed in a transcription-coupled fashion.

While the data concerning BER-mediated TCR appear to be somewhat contradictory, it is important to realize that preferential DNA repair depends on several factors: the type of damage, its location in the genome, and its ability to block RNA synthesis. A true understanding of the TCR pathway can only be obtained by considering the clearance of a variety of different adducts from specific loci in cells exhibiting different repair phenotypes. Indeed, in a somewhat unexpected way, the picture has been made even more complex by the discovery that mismatch recognition proteins comprise a pivotal element of TCR (*48*).

## The Role of Mismatch Repair

The genetics and biochemistry of methyl-directed MMR have been extensively characterized in *E. coli* (*2,9,68*). Single base mispairs and small heteroduplexes produced as a consequence of insertions or deletions of a few nucleotides in one strand of the duplex are corrected by this repair system. Strand discrimination, mismatch recognition, and incision require the participation of at least three proteins—MutS, MutL, and MutH. MutS binds heteroduplexes containing a mismatch, and MutH recognizes hemimethylated GATC sequences produced in newly replicated DNA. While no specific biochemical activity has been assigned to MutL, a model incorporating the roles of each of these proteins in MMR has been proposed. The coordinated action of MutS bound at a mismatch and MutL protein activates MutH endonuclease, resulting in the incision of the unmethylated strand near the GATC sequence. Since the nearest GATC sequence can be a considerable distance from the mismatch, assembly of the incision complex may be facilitated by the formation of a looped duplex to allow the direct interaction of MutS and MutH. In the presence of a preexisting nick in the duplex, correction can proceed in the absence of MutH and a GATC sequence, but MutS and MutL are still required. In addition to methyl-directed MMR, MutS and MutL also function in short patch repair of G–T mispairs and the processing of recombination intermediates.

The assortment of metabolic processes in which mismatch repair proteins participate was recently expanded to include

TCR. This was first documented studying the removal of CPDs from the individual strands of the induced *lac* operon in certain mutant strains of *E. coli* (*48*). Similar to previous observations in repair-proficient strains (*31*), CPDs were rapidly removed from the transcribed strand, while repair in the nontranscribed strand was much slower. Repair in *mutH*$^-$ strains was virtually identical to repair in wild-type strains. In contrast, mutations in either the *mutS* or *mutL* gene abolished the rapid repair of the transcribed strand of *lac*, and both strands were repaired at similar rates. These results were similar to those obtained studying repair in an *mfd* strain but are markedly different from repair in *uvr*$^-$ strains where no significant levels of repair in either strand of the *lac* operon were detected. These results suggest that in addition to Mfd and an RNA polymerase complex, MutS, MutL, and perhaps other factors are also involved in the coupling of NER and transcription.

The precise mechanism of TCR and the role of mismatch repair proteins in the process is unclear. Perhaps MutS and MutL function by recognizing some feature of the RNA polymerase complex stalled at a lesion or some structural distortion associated with the transcription bubble. The conformation of the DNA duplex on each side of the arrested complex may be altered, since transcription elongation appears to affect the topology. The spectrum of DNA substrates recognized by mismatch repair proteins has not been systematically tested. Some feature of the transcription bubble or the surrounding region might resemble a mismatched heteroduplex to which MutS binds and subsequently recruits MutL. Alternatively, MutS might directly bind CPDs. After MutS and MutL are recruited to the damaged site, they may promote the formation of a looped domain of DNA, similar to their proposed roles in mismatch repair. After the RNA polymerase complex is displaced by Mfd, the lesion is available for recognition and incision by the UvrA, UvrB, and UvrC proteins. In this model, recognition of the lesion by Uvr proteins is enhanced as a consequence of the altered DNA topology imposed by transcription elongation.

The link between mismatch repair and TCR could have important implications for human disease. Mutations in the human homologues of the *E. coli* mismatch repair genes have been associated with a common cancer predisposition syndrome, hereditary nonpolyposis colorectal cancer (HNPCC), and a subset of sporadic cancers (*69,70*). Recently, it has been demonstrated that several MMR-deficient human tumor cell lines and lymphoblastoid cell lines from HNPCC patients are also defective in TCR of CPDs (*71*). Thus, the connection between MMR and TCR first observed in *E. coli* extends to humans; furthermore, since mutations in MMR genes abolish TCR, it is possible that exposure to carcinogens and a reduction in the repair of environmentally induced damage contribute to the development of tumors associated with genetic defects in MMR.

The demonstration of a connection between MMR and TCR of CPDs raises several important questions: *a*) Are the MMR proteins responsible for the coupling of NER to transcription? *b*) When BER is coupled to transcription, does MMR play a role in the process? and *c*) Do deficiencies in TCR play a role in carcinogenesis? With regard to the final question, it is important to note that while cancer predisposition is not associated with CS, these patients die at very early ages.

## A Model for Transcription-coupled DNA Repair

Considering the complexity of TCR and the fact that the precise series of events that executes it is not clear, it might seem foolish to attempt to describe a model for the process; however, such an exercise provides a forum for addressing the issues that remain unclear and for asking pertinent questions. Figure 1 illustrates the fundamental steps of TCR as it might occur in humans and includes many of the components described in the previous sections. Following exposure to a genotoxic agent, damage to genes might occur. When such lesions are present in the noncoding—transcribed—strand, RNA synthesis can be impeded, provided that the actual adduct has an effect on RNA polymerase elongation. An important issue at this juncture concerns the effect of elongation factors on the behavior of the polymerase at a lesion; such factors might enhance bypass of the adduct either by increasing the RNA polymerase elongation rate or by permitting it to back up and try again, as is the case for $TF_{II}S$. Lesions that do not impede the polymerase either innately or because elongation factors assist in its bypass would escape TCR. This is complicated further by the fact that a subtle structural detail such as adduct stereochemistry can have a strong effect on the lesion's ability to block RNA polymerase. There is also the possibility that base misincorporation into the nascent transcript can facilitate pausing of RNA polymerase. In general, then, the first stage required for TCR, stalling of the RNA polymerase, is in and of itself affected by a complex array of factors, many of which still require further clarification of their particular roles in the process.

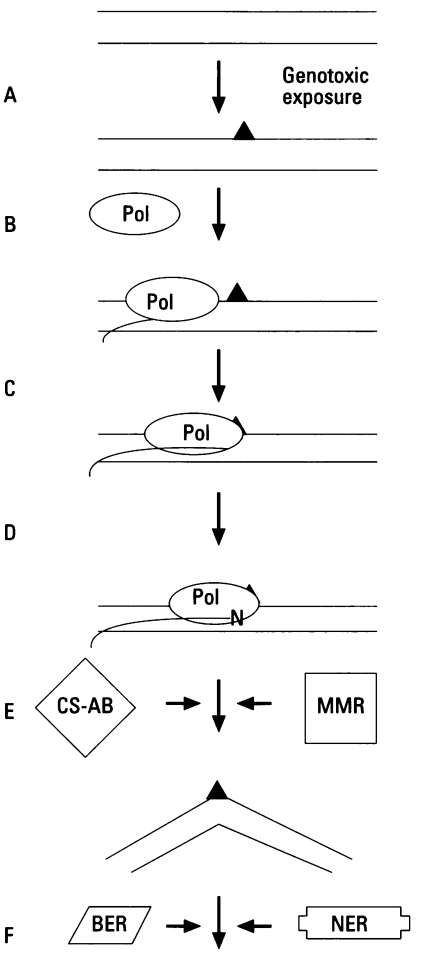


**Figure 1.** Model for TCR. (*A*) The organism is exposed to a genotoxin found in the environment; the agent produces damage in the genome as represented by the triangle. (*B*) Initiation of transcription occurs, and RNA polymerase (pol) enters elongation, using the noncoding strand as a template. (*C*) When the damage is present in the transcribed strand, it can decrease the processivity of RNA polymerase. (*D*) The polymerase can be stalled at the site of the adduct, perhaps in part by misincorporating an incorrect nucleotide, N, into the transcript in the vicinity of the adduct present on the noncoding strand. (*E*) The CS-AB proteins and the MMR proteins cause the damaged region to act as a better substrate for DNA repair pathways in the cell. (*F*) BER or NER, depending on the type of damage, removes the lesion, and following resynthesis of the DNA and ligation, repair is complete.

Once the RNA polymerase has stalled, CS-A, CS-B, and mismatch recognition proteins may enter the pathway. Precisely how these factors interact with DNA at or near the site of damage present on the transcribed strand remains unclear. At least two conceivable scenarios can be devised regarding the role of these proteins in TCR, both of which rely on the notion that they somehow convert the damaged site into a better substrate for repair enzymes. One possibility is that the CS-A and CS-B factors first displace the RNA polymerase in a manner equivalent to that of the Mfd protein in *E. coli*, making the lesion more available for recognition by the MMR proteins; this particular model relies on the prospect that the recognition system for MMR can also sense abnormal bases present in DNA, an event that has been documented for several DNA lesions (*72,73*). A second model involves the possibility that interactions among the CS-A, CS-B, and mismatch proteins change the supercoiled nature of the DNA at the adduct site, making the region better for recognition by repair proteins. Regardless of which of these models is correct—in reality, both may be proven to be incorrect—much biochemistry will need to be performed to test these prospects.

The final step in TCR concerns the precise nature of the repair pathway that ultimately clears the damage; as of now, either NER or BER participates in the process. While NER, BER, and MMR have been shown in part to effect TCR, the potential role of direct reversal repair pathways has yet to be elucidated. With regard to the repair machinery involved in TCR, more studies concerning the clearance of damage from cells with varying repair backgrounds is essential, particularly as mutants in BER become available. Also, the size of the repair patch following the TCR of a lesion from the transcribed strand of an expressed gene has not been established; hence no information concerning the involvement of long-patch versus short-patch repair can be used to support or refute any proposed mechanisms underlying TCR. Clearly, the general rules concerning TCR will emerge fully only after studies addressing these issues have been conducted.

It is clear that TCR operates on numerous types of DNA damage that can be induced in organisms by a wide range of environmental agents; indeed, it is becoming increasingly apparent that biased mutagenesis within genes can occur following exposure to chemicals and radiation, and it may well be a consequence of TCR at work (*2,3*). Because mutations in the genome of a cell can lead to transformation or death, an understanding of mechanisms that modulate cell formation of mutations is critical for comprehending how environmental carcinogens exert their toxic effects on an organism.

## REFERENCES


1. Singer B, Grunberger D. Molecular Biology of Mutagens and Carcinogens. New York:Plenum Press, 1983.
2. Friedberg EC, Walker GC, Siede W. DNA Repair and Mutagenesis. Washington:ASM Press, 1995.
3. Hanawalt PC. Transcription-coupled repair and human disease. Science 266:1957–1958 (1994).
4. Sancar A. Mechanisms of DNA excision repair. Science 266:1954–1956 (1994).
5. Lehmann AR. Nucleotide excision repair and the link with transcription. Trends Biochem Sci 20:402–405 (1995).
6. Van Houten B. Nucleotide excision repair in *Escherichia coli*. Microbiol Rev 54:18–51 (1990).
7. Sakumi K, Sekiguchi M. Structure and function of DNA glycosylases. Mutat Res 236:161–172 (1990).
8. Wallace SS. AP endonucleases and DNA glycosylases that recognize oxidative DNA damage. Environ Mol Mutagen 12:431–477 (1988).
9. Modrich P. Mismatch repair, genetic stability, and cancer. Science 266:1959–1960 (1994).
10. Pegg AE. Mammalian $O^6$-alkylguanine–DNA alkyltransferase: regulation and importance in alkylating carcinogenic and therapeutic agents. Cancer Res 50:6119–6129 (1990).
11. Sancar A. No "end of history" for photolyases. Science 272:48–49 (1996).
12. Hanawalt PC, Mellon I. DNA repair: stranded in an active gene. Curr Biol 3:67–69 (1993).
13. Scicchitano DA, Hanawalt PC. Intragenomic repair heterogeneity of DNA damage. Environ Health Perspect 98:45–51 (1992).
14. Bohr VA, Smith CA, Okumoto DS, Hanawalt PC. DNA repair in an active gene: removal of pyrimidine dimers from the *dhfr* gene of CHO cells is much more efficient than in the genome overall. Cell 40:359–369 (1985).
15. Mellon I, Bohr VA, Smith CA, Hanawalt PC. Preferential repair of an active gene in human cells. Proc Natl Acad Sci USA 83:8878–8882 (1986).
16. Mellon I, Spivak G, Hanawalt PC. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian *dhfr* gene. Cell 51:241–249 (1987).
17. Chen R-H, Maher VM, Brouwer J, van de Putte P, McCormick JJ. Preferential repair and strand-specific repair of benzo[*a*]pyrene diol epoxide adducts in the *HPRT* gene of diploid human fibroblasts. Proc Natl Acad Sci USA 89:5413–5417 (1992).
18. Carothers AM, Zhen W, Mucha J, Zhang Y-J, Santella RM, Grunberger D, Bohr VA. DNA strand-specific repair of (±)-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene adducts in the hamster dihydrofolate reductase gene. Proc Natl Acad Sci USA 89:11925–11929 (1992).
19. Tang MS, Qian M, Pao A. Formation and repair of antitumor antibiotic CC-1065-induced DNA adducts in the adenine phosphoribosyltransferase and amplified dihydrofolate reductase genes of Chinese hamster ovary cells. Biochemistry 33:2726–2732 (1994).
20. Leadon SA, Lawrence DA. Preferential repair of DNA damage on the transcribed strand of the human metallothionein genes requires RNA polymerase II. Mutat Res 255:67–78 (1991).
21. Stevnsner TA, May A, Petersen LN, Larminat F, Pirsel M, Bohr VA. Repair of ribosomal RNA genes in hamster cells after UV irradiation, or treatment with cisplatin or alkylating agents. Carcinogenesis 14:1591–1596 (1993).
22. Leadon SA, Cooper PK. Preferential repair of ionizing radiation-induced damage in the transcribed strand of an active human gene is defective in Cockayne syndrome. Proc Natl Acad Sci USA 90:10499–10503 (1993).
23. Vos J-M, Hanawalt PC. Processing of psoralen adducts in an active human gene: repair and replication of DNA containing monoadducts and interstrand cross-links. Cell 50:789–799 (1987).
24. Islas AL, Baker FJ, Hanawalt PC. Transcription-coupled repair of psoralen cross-links but not monoadducts in Chinese hamster ovary cells. Biochemistry 33:10794–10799 (1994).
25. Scicchitano DA, Hanawalt PC. Repair of *N*-methylpurines in specific DNA sequences in Chinese hamster ovary cells: absence of strand specificity. Proc Natl Acad Sci USA 86:3050–3054 (1989).
26. Wang W, Sitaram A, Scicchitano DA. 3-Methyladenine and 7-methylguanine exhibit no preferential removal from the transcribed strand of the dihydrofolate reductase gene in Chinese hamster ovary B11 cells. Biochemistry 34:1798–1804 (1995).

27. Tang MS, Bohr VA, Zhang XS, Pierce J, Hanawalt PC. Quantification of aminofluorene adduct formation and repair in defined DNA sequences in mammalian cells using UVRABC nuclease. J Biol Chem 264:14455–14462 (1989).
28. Tang MS, Pao A, Zhang XS. Repair of benzo[*a*]pyrene diol epoxide- and UV-induced DNA damage in dihydrofolate reductase and adenine phosphoribosyltransferase genes of CHO cells. J Biol Chem 269:12749–12754 (1994).
29. Sweder KS. Nucleotide excision repair in yeast. Curr Genet 27:1–16 (1994).
30. Snow ET. The role of DNA repair in development. Repro Toxicol (in press).
31. Mellon I, Hanawalt PC. Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. Nature 342:95–98 (1989).
32. Christians FC, Hanawalt PC. Inhibition of transcription and strand-specific DNA repair by α-amanitin in Chinese hamster ovary cells. Mutat Res 274:93–101 (1992).
33. Christians FC, Hanawalt PC. Lack of transcription-coupled repair in mammalian ribosomal RNA genes. Biochemistry 32:10512–10518 (1993).
34. Sauerbier W, Hercules K. Gene and transcription unit mapping by radiation effects. Annu Rev Genet 12:329–363 (1978).
35. Donahue BA, Yin S, Taylor JS, Reines D, Hanawalt PC. Transcript cleavage by RNA polymerase II arrested by a cyclobutane pyrimidine dimer in the DNA template. Proc Natl Acad Sci USA 91:8502–8506 (1994).
36. Shi Y, Gamper H, Hearst JE. Interaction of T7 RNA polymerase with DNA in an elongation complex arrested at a specific psoralen adduct site. J Biol Chem 263:527–534 (1988).
37. Shi Y, Gamper H, Hearst JE. The effects of covalent additions of a psoralen on transcription by *E. coli* RNA polymerase. Nucleic Acids Res 15:6843–6854 (1987).
38. Chen Y-H, Bogenhagen DF. Effects of DNA lesions on transcription elongation by T7 RNA polymerase. J Biol Chem 268:5849–5855 (1993).
39. Chen Y-H, Matsumoto Y, Shibutani S, Bogenhagen DF. Acetylaminofluorene and aminofluorene adducts inhibit *in vitro* transcription of a *Xenopus* 5S RNA gene only when located on the coding strand. Proc Natl Acad Sci USA 88:9583–9587 (1991).
40. Choi D-J, Marino-Alessandri DJ, Geacintov NE, Scicchitano DA. Site-specific benzo[*a*]pyrene diol epoxide–DNA adducts inhibit transcription elongation by bacteriophage T7 RNA polymerase. Biochemistry 33:780–787 (1994).
41. Selby CP, Sancar A. Gene- and strand-specific repair *in vitro:* partial purification of a transcription-repair coupling factor. Proc Natl Acad Sci USA 88:8232–8236 (1991).
42. Selby CP, Witkin EM, Sancar A. *Escherichia coli mfd* mutant deficient in "mutation frequency decline" lacks strand-specific repair: *in vitro* complementation with purified coupling factor. Proc Natl Acad Sci USA 88:11574–11578 (1991).
43. Lehmann AR. Three complementation groups in Cockayne syndrome. Mutat Res 106:347–356 (1982).
44. Mayne LV, Lehmann AR. Failure of RNA synthesis to recover after UV-irradiation: an early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. Cancer Res 42:1473–1478 (1982).
45. van Hoffen A, Natarajan AT, Mayne LV, van Zeeland AA, Mullenders LHF, Venema J. Deficient repair of the transcribed strand of active genes in Cockayne's syndrome cells. Nucleic Acids Res 21:5890–5895 (1993).
46. Troelstra C, Odijk H, de Wit J, Westerveld A, Thompson LH, Bootsma D, Hoeijmakers JHJ. Molecular cloning of the human DNA excision repair gene ERCC-6. Mol Cell Biol 10:5806–5813 (1990).
47. Henning KA, Li L, Iyer N, McDaniel LD, Reagan MS, Legerski R, Schultz RA, Stefanini M, Lehmann AR, Mayne LV, Friedberg EC. The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II $TF_{II}H$. Cell 82:555–564 (1995).
48. Mellon I, Champe GN. Products of DNA mismatch repair genes *mutS* and *mutL* are required for transcription-coupled nucleotide-excision repair of the *lactose* operon in *Escherichia coli.* Proc Natl Acad Sci USA 93:1292–1297 (1996).
49. Cleaver JE, Kraemer KH. Xeroderma pigmentosum. In: The Metabolic Basis of Inherited Disease (Scriver CR, Beaudet AL, Sly WS, Valle D, eds). New York:McGraw Hill Book Co, 1989;2949–2971.
50. Jones CJ, Wood RD. Preferential binding of the xeroderma pigmentosum group A complementing protein to damaged DNA. Biochemistry 32:12096–12104 (1993).
51. Weeda G, van Ham RCA, Masurel R, Westerveld A, Odijk H, de Wit J, Bootsma D, van der Eb AJ, Hoeijmakers JHJ. Molecular cloning and biological characterization of the human excision repair gene *ERCC-3.* Mol Cell Biol 10:2570–2581 (1990).
52. Legerski R, Peterson C. Expression cloning of a human DNA repair gene involved in xeroderma pigmentosum group C. Nature 359:70–73 (1992).
53. Weber CA, Salazar EP, Stewart SA, Thompson LH. *ERCC2*: cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast *RAD3.* EMBO J 9:1437–1447 (1990).
54. Weber CA, Salazar EP, Stewart SA, Thompson LH. Molecular cloning and biological characterization of a human gene, *ERCC2*, that corrects the nucleotide excision repair defect in CHO UV5 cells. Mol Cell Biol 8:1137–1146 (1988).
55. Chu G, Chang E. Xeroderma pigmentosum group E cells lack a nuclear factor that binds to damaged DNA. Science 242:564–567 (1988).
56. Thompson LH, Brookman KW, Weber CA, Salazar EP, Reardon JT, Sancar A, Deng Z, Siciliano MJ. Molecular cloning of the human nucleotide excision repair gene *ERCC4.* Proc Natl Acad Sci USA 91:6855–6859 (1994).
57. O'Donovan A, Wood RD. Identical defects in xeroderma pigmentosum group G and rodent ERCC group 5. Nature 363:185–188 (1993).
58. van Duin M, Vredeveldt G, Mayne LV, Odijk H, Vermeulen W, Klein B, Weeda G, Hoeijmakers JHJ, Bootsma D, Westerveld A. The cloned human DNA excision repair gene *ERCC-1* fails to correct xeroderma pigmentosum complementation groups A through I. Mutat Res 217:83–92 (1989).
59. Evans MK, Robbins JH, Ganges MB, Tarone RE, Nairn RS, Bohr VA. Gene-specific DNA repair in xeroderma pigmentosum complementation groups A, C, D, and F. J Biol Chem 268:4839–4847 (1993).
60. Hoeijmakers JHJ. Nucleotide excision repair II: from yeast to mammals. Trends Genet 9:211–217 (1993).
61. Schaeffer L, Roy R, Humbert S, Moncollin V, Vermeulen W, Hoeijmakers JHJ, Chambon P, Egly J-M. DNA repair helicase: a component of BTF2 ($TF_{II}H$) basic transcription factor. Science 260:58–63 (1993).
62. Mansbridge JN, Hanawalt PC. Domain-limited repair of DNA in ultraviolet irradiated fibroblasts from xeroderma pigmentosum complementation group C. In:Cellular Responses to DNA Damage (Friedberg EC, Bridges BA, eds). New York:Alan R Liss Inc, 1983;195–207.
63. Kantor GJ, Elking CF. Biological significance of domain-oriented DNA repair in xeroderma pigmentosum cells. Cancer Res 48:844–849 (1988).
64. Kantor GJ, Barsalou LS, Hanawalt PC. Selective repair of specific chromatin domains in UV-irradiated cells from xeroderma pigmentosum complementation group C. Mutat Res 235:171–180 (1990).
65. Venema J, van Hoffen A, Karcagi V, Natarajan AT, van Zeeland AA, Mullenders LHS. Xeroderma pigmentosum complementation group C cells remove pyrimidine dimers selectively from the transcribed strand of active genes. Mol Cell Biol 11:4128–4134 (1991).
66. Scicchitano DA, Hanawalt PC. Lack of sequence-specific removal of *N*-methylpurines from cellular DNA. Mutat Res 233:31–37 (1990).
67. Engelward B, Dreslin A, Christensen J, Huszar D, Kurahara C,

Samson L. Repair deficient 3-methyladenine DNA glycosylase homozygous mutant mouse cells have increased sensitivity to alkylation induced chromosome damage and cell killing. Eur Mol Biol Organ J 15:945–952 (1996).
68. Modrich P. Mechanisms and biological effects of mismatch repair. Annu Rev Genet 25:229–253 (1991).
69. Kolodner RD. Mismatch repair: mechanisms and relationships to cancer susceptibility. Trends Biochem Sci 20:397–401 (1995).
70. Kunkel TA. The intricacies of eukaryotic spell-checking. Curr Biol 5:1091–1094 (1995).
71. Mellon I, Rajpal DK, Koi M, Boland CR, Champe GN. Transcription-coupled repair deficiency and mutations in human mismatch repair genes. Science 272:557–360 (1996).
72. Duckett DR, Drummond JT, Murchie AIH, Reardon JT, Sancar A, Lilley DMJ, Modrich P. Human MutSα recognizes damaged DNA base pairs containing $O^6$-methylguanine, $O^4$-methylthymine, or the cisplatin-d(GpG) adduct. Proc Natl Acad Sci USA 93:6443–6447 (1996).
73. Mello JA, Acharya S, Fishel R, Essigmann JM. The mismatch-repair protein hMSH2 binds selectively to DNA adducts of the anticancer drug cisplatin. Chem Biol 3:579–589 (1996).